Development of a spontaneously beating vein by cardiomyocyte transplantation in the wall of the inferior vena cava in a rat: A pilot study

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Purpose: **This study was conducted to determine whether it is feasible to develop a vein that rhythmically beats by implanting immature cardiomyocytes in its wall.**

Methods: **Neonatal cardiomyocytes (5×10^6 cells each) were transplanted into the wall of the inferior vena cava in six female Fischer rats; in six rats, only the medium was transplanted. At 3 weeks after transplantation, the grafted site of the inferior vena cava was exposed and videotaped, and then processed for histology.**

Results: **Distinct rhythmic beating of the vena cava at the site of cell injection (at a rate lower than aortic beating) was observed in all six rats treated with neonatal cardiomyocyte injections, but in none of the six that received the medium. The vena cava continued to beat spontaneously and rhythmically after the aortas were clamped and after the heart was excised. The beating was manifest by visual contraction and relaxation of the vessel wall. The spontaneous beating rate was 101 ± 7 beats/min at 1 to 3 minutes after excision of the heart. Hematoxylin and eosin staining showed viable grafts in the wall of the vena cava in all that were implanted with neonatal cardiac cells; but in none of the vena cava that received the medium. Neonatal cardiomyocytes in the graft matured with cross striations and stained positive for the muscle marker sarcomeric actin.**

Conclusions: **The present study demonstrates that neonatal cardiomyocytes survive, mature, and spontaneously and rhythmically contract when implanted in the wall of a vein. (J Vasc Surg 2007;45:817-20.)**

Clinical Relevance: **Venous thrombosis occurs in approximately 0.1% of the general population each year in the United States. The interaction between vascular endothelium and platelets plays a key role in the thrombotic event. Pulsatility has been hypothesized to affect the function of vascular endothelium and platelet adhesion. Our present study demonstrates that engrafted neonatal cardiac cells within the wall of the rat inferior vena cava survive, develop an adult phenotype, and exhibit spontaneous beating. This model provides a useful experimental approach to investigate the effect of venous pulsatility on the vascular endothelial behavior in vivo and to determine whether pulsatility prevents platelet adhesion to endothelium in venous stasis and, ultimately, protects against venous thrombosis.**

Venous thrombosis occurs in approximately 0.1% of the general population each year in the United States.[1] The underlying etiologic factors for venous thrombosis involve Virchow's triad for thrombogenesis, including vascular damage to the vein wall,[2] venous stasis,[3] and hypercoagulability of blood.[4] Among the multifactorial etiologies, venous stasis is important in the thromboembolic process. Venous thrombosis typically develops in regions of slow blood flow, for example, in valve cusp pockets. Reduced blood flow causes stagnant blood pools in which activation products of the coagulation system accumulate, leading potentially to local hypercoagulability. The distension of the vessel walls induced by the pooling of blood can also result in endothelial cell damage. Rhythmic mechanical compression has been demonstrated to prevent venous stasis and venous thrombosis.[5]

Implantation of cardiac cells around the vein might serve as a suitable fatigue-resistant power source for venous compression. We have previously transplanted neonatal cardiac cells into the wall of the abdominal aorta in rats and demonstrated that the implanted immature neonatal cardiomyocytes survived, matured, developed a blood supply, and spontaneously contracted and generated measurable contractile force within the wall of the aorta in rats.[6,7] However, whether neonatal cardiomyocytes could even survive in the environment of a venous wall is unknown. In this study, we investigated whether neonatal cardiac cells can survive, mature, and spontaneously contract in the wall of the inferior vena cava in rats.

MATERIALS AND METHODS

The Association for Assessment and Accreditation of Laboratory Animal Care International accredits Good Samaritan Hospital. All procedures performed on animals were approved by the Institutional Animal Care and Use Committee of Good Samaritan Hospital and were in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 85-23, National Academy Press, Washington DC, revised 1996).

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Cell isolation and purification. Neonatal ventricular cardiomyocytes were isolated from the neonatal hearts of Fischer CDF rats (2 days after birth) and cultured by previously described methods.[8] In brief, after removal of the atria and great vessels, the ventricles were minced into small fragments (1 mm^3). The minced ventricles were incubated in digestion buffer containing 100 U/mL collagenase and 0.6 mg/mL pancreatin. The isolated cells were purified by preplating 30 minutes at 37°C to eliminate attached cells, as previously described.[8] The nonattached cells were plated in culture dishes and cultured in the presence of 5% carbon dioxide and 95% air at 37°C for 1 to 2 days before transplantation.

Cell transplantation into the wall of the inferior vena cava. Female Fischer CDF rats were anesthetized with intraperitoneal ketamine (75 mg/kg) and xylazine (5 mg/kg), and placed in the supine position. The abdominal area was shaved and prepared for surgery. A midline incision was made, and the inferior vena cava was exposed after the intestine was retracted into the right portion of the abdomen. The site of injection was approximately 3 mm below the level of the origin of renal veins. The fascia around the vein was dissected free at this site. The inferior vena cava was adequately exposed, and neonatal cardiomyocytes (5×10^6 cells each) were transplanted into six rats as described below, and six rats received medium.

Injections were performed with a 28-gauge needle attached to an insulin syringe under magnification with a dissecting microscope. Approximately 150 μL of base methylcellulose medium in Iscove modified Dulbecco media (Stemcell Technologies, Inc, Vancouver, British Columbia, Canada), with or without cells, was injected directly into the outer wall of the inferior vena cava and encompassing its entire circumference to form a cuff. An even and homogenous grafted cuff containing medium or cells around the inferior vena cava was formed by using four injection sites around the circumference: anterior, left lateral, right lateral, and posterior. The posterior injection was accomplished by twisting the inferior vena cava back to front. After successful injection, a raised pale bleb extending 2 to 3 mm along the length of the inferior vena cava and protruding about 2 mm above the natural surface of the inferior vena cava was observed. After the injection was completed, the incision was closed by suturing the muscle and stapling the skin. Buprenex (0.001 mg/100 g body weight, twice a day; Reckitt Benckiser Pharma, Richmond, Va) was given subcutaneously for 2 days as an analgesic after surgery.

Contractile evaluation. At 3 weeks after surgery, the rats were anesthetized with ketamine/xylazine and the inferior vena cava was re-exposed at the site where the cells (or medium) were implanted. The grafted cuff around the inferior vena cava was isolated by careful dissection. Heparin (300 U/kg) was administered intravenously to the rats. The spontaneous beating of grafted cardiomyocytes was examined and documented by video camera before and after the heart was excised. The spontaneous beating rate (beats/min) was counted on the video recording by the previously described procedures.[6]

Histologic and immunohistochemical examination. After contractile evaluation, the inferior vena cava was removed, washed in 0.9% saline, and fixed in 10% formaldehyde for histology. Paraffin-embedded samples were cut into 5-μm sections for hematoxylin and eosin staining and immunohistochemical staining with primary antibody against sarcomeric actin (1:75 M0874, Dako, Carpinteria, Calif).

Statistical analysis. All results are presented as mean ± standard error of the mean. Difference between groups was compared by the Student *t* test. Statistically significant difference was established at $P < .05$.

RESULTS

Contractile potential. Contractile capability was determined before and after the heart was excised at 3 weeks. The in vivo heart rate assessed by aortic pulsation after ketamine/xylazine anesthesia was 141 ± 7 beats/min. Distinct rhythmic beating of the vena cava at the site of cell injection, at a rate of 92 ± 6 beats/min, was significantly lower than the rate of aortic beating ($P = .002$) and was observed in all six rats injected with neonatal cardiomyocytes but in none of the six animals that received media. The vena cava continued to beat spontaneously and rhythmically after the aortas were clamped and the hearts were excised. The beating was manifest by visual contraction and relaxation of the vessel wall. The spontaneous beating rate was 101 ± 7 beats/min at 1 to 3 minutes after excision of the heart. No significant difference was noted compared with the rate before heart excision (see video clip, online only).

Histology. At 3 weeks after surgery, hematoxylin and eosin staining showed viable grafts around the wall in all six of the cell-treated inferior vena cavae (Fig 1, *A* and *C*) and in none of the six inferior vena cavae receiving medium (Fig 1, *B* and *D*). Neonatal cardiomyocytes in the graft formed compact, longitudinally oriented cardiac muscle bundles and mature cross striations (sarcomeres). Most of the grafted cardiomyocytes were organized into a circular pattern parallel to the vein layers (Fig 1, *C*). The structure of the vein wall at the graft site was normal in all samples.

Immunohistochemical staining of the inferior vena cava for sarcomeric actin was positive in all six cell-treated inferior vena cavae (Fig 2) and in none of the inferior vena cava in the medium group after surgery. The striated cells were easy to identify within the wall of the inferior vena cava.

DISCUSSION

Our present data demonstrate for the first time, to our knowledge, that engrafted neonatal cardiac cells around the outer wall of the rat inferior vena cava survive, develop an adult phenotype with cross striation, and exhibit spontaneous contractions.

In animal studies, cardiac cells have been transplanted into ears,[9] skeletal muscle,[10] subcutaneous connective tis-

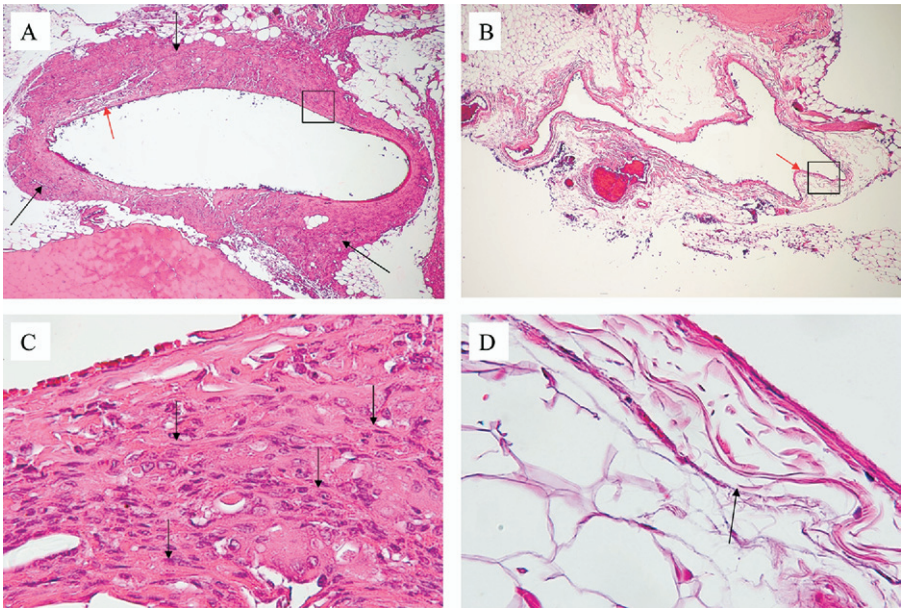

Fig 1. Representative hematoxylin and eosin stained sections of the abdominal inferior vena cava. **A,** Inferior vena cava that received neonatal heart cells. Viable grafts *(black arrows)* are located around the inferior vena cava *(red arrow)*. **B,** Inferior vena cava in a control rat that received medium only. The wall of the inferior vena cava *(red arrow)* is surrounded by loose connective tissue (*A* and *B*, original magnification ×40). **C,** Higher magnification (×400) of the boxed area in *A*. The transplanted cardiomyocytes *(black arrows)* are visible in the outer wall of the vena cava. **D,** Higher magnification (×400) of the boxed area in *B* shows the loose connective tissues around the vena cava *(black arrow)*.

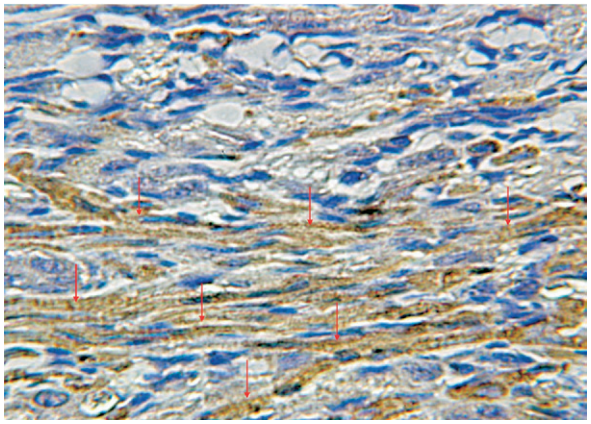

Fig 2. Immunohistochemical staining of the same slices as in Fig 1, *A* for sarcomeric actin. The grafted cardiomyocytes are organized in a circular pattern parallel to the vena cava wall, and show stronger positive brown staining and typical cross striations of sarcomere formation *(red arrows)* (×400).

sue,[11] myocardial infarctions,[12] and within the wall of the aorta.[6,7] In 2006, we reported that engrafted cardiomyocytes within the aortic wall generated 0.36 ± 0.05 mm Hg pulse pressure without pacing; during pacing, the pulse pressure was 0.78 ± 0.21 mm Hg, with systolic pressure up to 3.8 mm Hg.[7] Our recent unpublished data demonstrated that cardiomyocytes survived as long as 10 months within the rat aortic wall and form a circumferential cuff within the rat aortic wall. In this study, transplanted cardiac cells kept a vein rhythmically beating. Thus, as a fatigue-resistant power source, transplanted cardiac cells have the potential to provide biologic power for pulsatile motion of the vein.

One method of prophylaxis against venous stasis and thrombosis is mechanical external compression, such as compression stockings[13] and intermittent compression devices.[14,15] The pulsing venous wall motion induced by rhythmically contracting transplanted cardiac cells might be potentially used to prevent vein thrombosis. The rhythmically beating vein may generate pulsatile flow within the lumen.

Flow conditions can influence the antithrombotic and prothrombotic responses of vascular endothelium.[16] Compared with nonpulsatile flow, pulsatile flow significantly enhanced saphenous venous endothelial cell nitric oxide production in a three-dimensional hemodynamically active model.[17] Flow pulsatility may influence the platelet adhesion to endothelium. Jen et al[18] demonstrated that platelets attached on a fibrinogen-coated surface in a tapered flow chamber were more effectively removed by pulsatile flow than by equivalent steady flow.

In a clinical study, Naschitz et al[19] observed that five patients with pulsatile venous insufficiency caused by severe tricuspid regurgitation did not develop venous thrombosis, phlebitis, or cutaneous ulcers during an average 9.4-year follow (range, 6 to 15 years). Patients with pulsatile venous insufficiency had a better clinical course, diminished leuko-

cyte trapping, and increased flow in the distal calf veins compared with patients with nonpulsatile venous insufficiency. These authors suggested that pulsatile shear stress enhances secretion of cytokines of venous endothelial cells that prevent a predisposition to platelet aggregation, hypercoagulability, and white cell adhesion.

Thus, in theory, the rhythmically beating cardiomyocytes within the vein wall could become a potential power source for rhythmic contraction of veins to help to prevent venous thrombosis. This might be relevant to patients who are immobilized by paralysis, orthopedic procedure, or disease such as end-stage cancer.

CONCLUSION

This pilot study demonstrated that transplantation of immature neonatal cardiomyocytes into the wall of a vein has the potential to cause rhythmic pulsations in the vein wall. This model provides a useful experimental approach to investigate the effect of venous pulsatility on the vascular endothelial behavior in vivo and to determine whether pulsatility prevents platelet adhesion to endothelium in venous stasis and protects against vein thrombosis. Future studies are needed to measure the strength of contraction, diameter reduction, as well as flow velocity in the beating vein.

AUTHOR CONTRIBUTIONS

Conception and design: WD, SLH, RAK
Analysis and interpretation: WD, SLH, RAK
Data collection: WD, SLH, RAK
Writing the article: WD, SLH, RAK
Critical revision of the article: WD, SLH, RAK
Final approval of the article: WD, SLH, RAK
Statistical analysis: WD, SLH, RAK
Obtained funding: RAK
Overall responsibility: RAK

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